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Reduction of microbial and faunal groups following application of streptomycin and captan in Georgia no-tillage agroecosystems

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With 2 figures

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1. Introduction

Organic matter reserves in agricultural soils can be severely depleted by years of soil tillage. This depletion is clearly related, in part, to accelerated decomposition rates, but the implications of more rapid decomposition rates are not fully understood. Increased mixing of residues into the soil likely results in changes in soil foodweb structure and function following soil disturbance (HENDRIX *et al.*, 1990). Investigation of this hypothesis was performed by manipulating key components of the detrital food web in agroecosystems with chemical biocides.

Many taxonomic groups belonging to the belowground food web have been studied in Georgia agricultural soils but the role of the entire soil food web with respect to nutrient cycling has only been hypothesized (HENDRIX *et al.*, 1986). A useful tool in determining the role of component groups in food web function is the use of biocides, although care must be taken in interpreting results from biocide studies (INGHAM & COLEMAN, 1984).

The direct effect of each biocide on each functional group should be determined. Pesticide literature indicates that biocides are neither equally effective on all populations within a taxonomic group, not do they affect target and non-target organisms in similar manners when applied to different soils or in different climatic/vegetations regimes (INGHAM & COLEMAN, 1984). Therefore, each biocide should be tested in each new soil and climate to improve interpretive ability of the effect of the biocide on each component group in the soil food web.

Biocides which remove or reduce component groups of the soil food web have been successfully utilized in deserts (SANTOS & WHITFORD, 1981, PARKER *et al.*, 1984), a semiarid grassland (INGHAM & COLEMAN 1984, INGHAM *et al.* 1986), meadow and forest (INGHAM *et al.*, 1986), and in semiarid agro-ecosystems (L. PORTER, pers. comm.). Reduction of the various food web component populations allowed determination of their functions in nutrient cycling and elucidated interactions between functional groups.

The effects of streptomycin sulfate and captan on target and non-target organisms in no-till Georgia agroecosystems were determined. These initial biocide tests were performed in small plots near the long-term experimental fields to evaluate biocides effective in reducing their target organism group, yet have the fewest effects on non-target organisms. Field tests allowed the biocides to be evaluated under the abiotic conditions normal for further field trials.

2. Materials and methods

These studies were performed at the Horseshoe Bend Experimental Area at the University of Georgia, Athens, Georgia, where comparisons of till and no-till agriculture have been maintained for twelve years. The Horseshoe Bend site is characteristic of agriculture in Georgia river-bottom soil

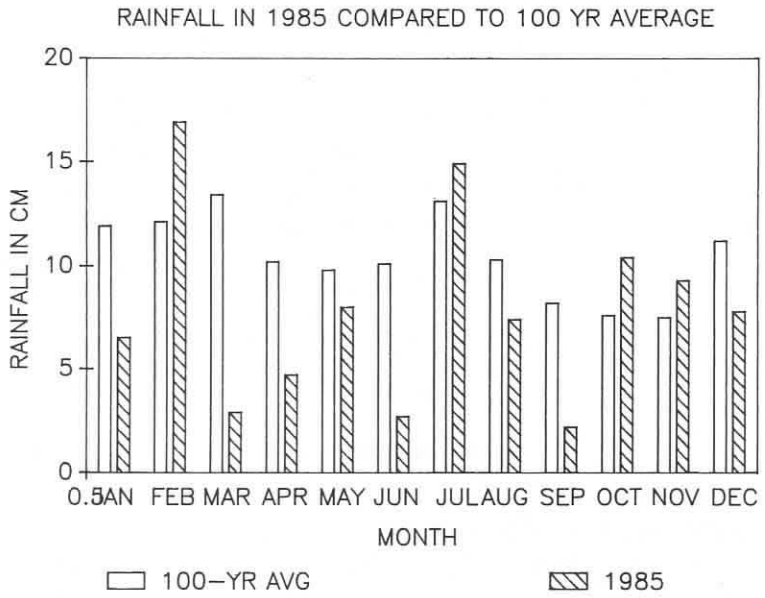

Fig. 1. Monthly rainfall at Horseshoe Bend, the experimental sites, near Athens, Georgia in 1985 as compared to 100 year average rainfall levels.

(Typic Rhodudult, table 1). The experiments were carried out during the late summer (August 19 to September 18) of 1985. Temperatures were typical for Georgia during this time, ranging between 18 and 25 °C, with an average of 22 °C. Precipitation was uncharacteristically low during most of the summer before the experiment (figure 1), and in fact, this was the beginning of a three year drought (PERDUE & CROSSLEY, 1990).

To determine which biocides were most effective in reducing groups of organisms with minimal non-target effects, these initial studies were performed in no-till plots only. The greater amount of litter and organic matter in no-till, as compared to tilled plots, increases the adsorption of biocides and reduces their effects (STEVENSON, 1985). Thus, if a biocide is capable of producing an effect in the no-till plots, it is reasonable to expect the biocide to be equally, and possibly more, effective in the tilled plots.

Based on recommended field application rates, three concentrations of both captan (fungicide) and streptomycin (bactericide) were applied on August 19, or August 21, 1985, respectively (see table 2 for dosages). Five plots for each biocide concentration were established in an old-field grass sward converted to no-till plots. Plots were enclosed in polyacrylic plastic sheets placed 10 cm into the ground, extending 20 cm above ground. Soil inside the plots were brought to field capacity with suspensions of biocides in water (table 2). Biocide concentrations was based on the water suspensions reaching

Table 1. Soil characteristic of till and no-till soils at Horseshoe Bend, University of Georgia Agroecosystem Study Site, Athens, Georgia.

Depth (cm)	Organic Carbon (kg ha^{-1})	Organic Nitrogen (kg ha^{-1})	Bulk Density (g cm^{-3})	CEC	pH
Conventional till					
0– 5	9067	849	1.40	4.86	5.9
5–13	11481	1306	1.49	4.70	6.0
No-till					
0– 5	14155	1201	1.21	7.77	6.4
5–13	10672	1234	1.51	3.30	5.5

Table 2. Biocide concentrations, target organism, and application dates in each experiment.

Biocide Name	Target organism	Concentration	Application date
Streptomycin Sulfate	Bacteria	0.1 mg per 1.0 gram soil 5.0 gram soil 10.0 gram soil	August 19, 1985
Captan	Fungi	25 µg per gram soil	August 21, 1985

a 15 cm soil depth. Three replicate 2 cm diameter, 10 cm deep soil cores were taken on day 1, 3, 7, 14, and 30 after application from each plot. Three separate cores of the same size were collected for nematode extraction on days 7 and 14. Microarthropods were sampled with a 5 cm × 5 cm core on day 14 only. On each sample date, the soil cores were divided into litter, 0 – 5, and 5 – 10 cm depths. Abiotic conditions were monitored throughout each experiment (table 3), and gravimetric soil moisture was measured routinely.

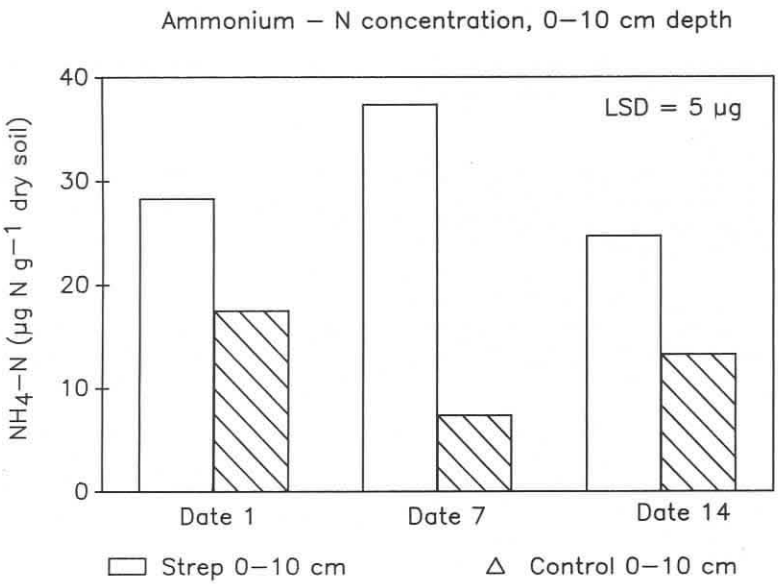

Fig. 2a. Ammonium-N concentration in streptomycin and streptomycin control soils 0 – 10 cm depths. Three way interaction of depth, date, and biocide significant at $P < 0.031$. LSD = 5 µg per gram soil.

The following assays were performed:

(1) Total numbers of bacteria determined using FITC (fluorescein isothiocyanate) direct estimations (BABIUK & PAUL, 1970). Ten-fold dilutions using an initial 1 gram soil were prepared. One ml of each dilution was stained with FITC, destained with pyrophosphate and buffer solutions the filters placed on slides and the number of fluorescent bacteria in each of ten fields on each filter counted. That dilution with a bacterial count between 25 and 75 was used to give the best estimate. On succeeding sample dates, that dilution was initially prepared and if numbers of bacteria were between 25 and 75, that estimate was considered appropriate. If numbers were too low or too high, another dilution, higher or lower as the case indicated, was chosen for bacterial enumeration;

(2) Active and total hyphal lengths using FDA (fluorescein diacetate) staining (INGHAM & KLEIN, 1984). Ten fold dilutions, using an initial 1 gram of soil from each sample, were prepared. One ml aliquots from each 1 : 10 or 1 : 100 dilution was stained with FDA for at least 3 minutes,

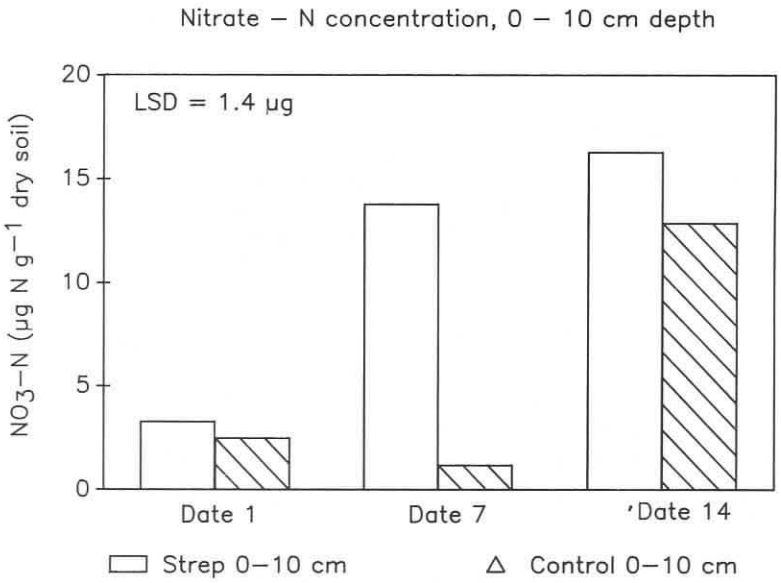

Fig. 2b. Nitrate-nitrite-N concentrations in streptomycin and streptomycin control soils 0–10 cm depths. Three way interaction of depth, date, and biocide significant at $P < 0.003$. LSD = 0.7 µg per gram soil.

filtered to remove excess stain, agar added to the re-suspended soil and placed on a known volume well on a slide. Length of active (FDA-stained) and total hyphae were determined:

(3) Most Probable Numbers of protozoa were determined by plating four or six 0.5 ml aliquots from each ten-fold dilution (10–1 to 10–6 dilutions) in microtiter wells containing soil extract agar (DARBYSHIRE *et al.*, 1974). Suspensions were incubated at room temperature for 7–14 days to allow protozoan growth, at which time aliquots from each well were observed for the presence or absence of each protozoan group, flagellates, amoebae or ciliates;
(4) Extraction of nematodes using Baermann funnels for 48 hours (ANDERSON & COLEMAN, 1977);
(5) Extraction of microarthropods for 1 week on MERCHANT & CROSSLEY (1970) high gradient Tullgren extractors;
(6) Inorganic nitrogen concentrations (HENDRIKSEN & SELMER-OLSEN, 1970).

A three way analysis of variance was performed, using date, depth, and biocide as treatment variables (NIE *et al.*, 1975). Only those main or interactive effects indicated significant by the F test at $P < 0.05$ are discussed. The mean separation test utilized was Least Significant Differences (LSD) calculated for a significance level of $P < 0.01$ (F-protected LSD).

3. Results

3.1. General

In 1986, weather in Georgia was unusually dry (figure 1). Biocides were applied in water, increasing moisture levels to field capacity. Thus initial activity levels of organisms were likely increased because of the added moisture, but were expected to decrease as the soils dried.

3.2. Streptomycin and captan

3.2.1. Bacteria

Litter – Total bacterial numbers were not reduced by streptomycin. Bacterial numbers remained near 1×10^8 bacteria g^{-1} litter in all treatments throughout the experiment, except

in one instance. A significant increase in the number of bacteria in the captan-treated litter occurred 14 days after captan application, an increase from 1×10^8 to 8.9×10^9 bacteria g^{-1} litter (LSD = 2.3×10^8 g^{-1} litter). Otherwise, both treatments were not significantly different.

Soil, 0–5 cm depth – Total bacterial numbers were significantly reduced from 6.3×10^8 to 3.2×10^8 g^{-1} soil seven days after streptomycin application. By day 14, no difference in numbers between control and streptomycin treatments was detectable, with both treatments near 6.2×10^8 g^{-1} soil. Captan teated soils were not significantly different from controls at any time, with numbers in both soils remaining between 5 and 7×10^8 g^{-1} soil throughout the experiment.

3.2.2. Active fungi

Litter – Active (FDA-stained) hyphal lengths were significantly reduced by streptomycin on the first sample date (10 meters as compared to 24 meters g^{-1} litter in controls), but hyphal lengths returned to control levels by the second sample date. However, if the captan control and streptomycin control were averaged, no treatment effect would have been noted. This suggests that the samples removed from the streptomycin control plot contained aberrantly high levels of fungal biomass. In the captan treatment, active hyphae in litter were significantly lower than in controls on day 7,2 m as compared to 9 m in controls (LSD = 3.6 m), and were still lower on day 30.

Soil, 0–5 cm depth – Streptomycin had no effect on fungal activity, while in captan treated soil, activity was reduced on day 7 (2 m as compared to 6.2 m g^{-1} soil in controls, LSD = 3.6 m). However, by day 14, the activity of fungi in captan-treated soils was 3-fold greater than in controls (9.1 m as compared to 3.2 m). By day 30, active hyphal length had returned to control levels in the captan-treated soil.

3.2.3. Total fungi

Litter – There were no significant differences between streptomycin and control, or captan and control treatments throughout the experiments, suggesting that empty fungal hyphae are some-what recalcitrant nutrient sources and decompose slowly. Fluctuations in fungal dynamics are often masked by this pool of empty fungal hyphae. Total hyphal lengths averaged 400 m g^{-1} soil, LSD = 143 m in both soils.

Soil, 0–5 cm depth – Total hyphal length in streptomycin treated soils was not significantly different from controls, averaging 200 m g^{-1} soil. In the captan treatment, total hyphae responded in a similar fashion to that seen for active hyphae. Levels of total hyphae were lower on day 7 (40 as compared to 120 m g^{-1} soil in controls), were higher on day 14 (270 m as compared to 120 m g^{-1} in controls), and were not significantly different on days 30 (average of 120 m g^{-1} soil in controls, streptomycin, and captan treated soils).

3.2.4. Protozoa

Litter – Flagellate numbers were higher in the streptomycin treatment (9×10^5 g^{-1} litter) as compared to controls (8×10^3 g^{-1} litter, LSD = 7×10^4). There were no significant differences between numbers of flagellates (3×10^3 vs 2×10^4 g^{-1} litter) in the control and captan treatments. No significant differences in numbers of amoebae (10^3 to 10^4 g^{-1} litter) or ciliates (5×10^3 and 1×10^4 g^{-1} litter) were noted between any treatment and controls.

Soil, 0–5 cm depth – There was no significant difference between streptomycin, captan, or either control treatment with respect to flagellates in 0–5 cm depths, or amoebal or ciliate numbers in either litter or soil. Amoebal and ciliate numbers tended to decrease with

depth. Amoebae ranged from 10^3 to 10^4 g^{-1} litter, from 2×10^2 to 3×10 g^{-1} 0–5 cm soil, and from 50 to 5×10^3 g^{-1} in the 5–10 cm soil depth. Ciliates ranged between 5×10^3 and 1×10^4 g^{-1} litter, 1×10^2 and 3.5×10^2 and 3.5×10^2 g^{-1} 0–5 cm soil, and between 50 and 5×10^2 g^{-1} in 5–10 cm soil.

3.2.5. Nematodes

Soil, 0–5 cm depth – Both bacterial- and fungal-feeding nematode numbers were not affected by streptomycin treatment (numbers remained between 8 and 20 g^{-1} soil). Fungal-feeding nematodes were significantly reduced by captan treatment on day 14, (4 as compared to 13 g^{-1} soil in controls).

Soil, 5–10 cm depth – While numbers of both bacterial- and fungal-feeding nematodes were 2-fold lower at 5–10 cm depths than in 0–5 cm depths, neither biocide treatment had a significant effect on these nematodes in these soils as compared to controls.

Captan and streptomycin had little effect on omnivorous, or plant-feeding nematodes (7 g^{-1} soil). The only significant effect was an increase in plant-feeding nematodes in the captan treatment to 19 nematodes g^{-1} in the 5–10 cm depth on day 14 as compared to 10 in controls.

3.2.6. Microarthropods

Only Collembola were significantly effected by captan, with no effects of streptomycin on any arthropod group. Initial numbers of collembola were between 5600 and 6600 per m^2 in all treatments and remained at this level in control soils. In captan treated soil, Collembola numbers increased to 11,200 per m^2 on day 7, but returned to control ranges by day 14.

3.2.7. Nitrogen

Nitrogen concentrations were measured only in soil samples, not in litter. Ammonium-N concentration in 0–5 cm and 5–10 cm soil depths were significantly increased in the streptomycin treatment (fig. 2). Nitrate-nitrite-N also significantly increased with time in the streptomycin treatment in both soil depths (fig. 3). Captan did not affect inorganic N levels as compared to controls.

4. Discussion

In these Georgia soils streptomycin decreased bacterial numbers for a very short period of time, less than 14 days in the soil, but not at all in the litter. As compared to a several month duration, 50 to 75% decrease in bacterial numbers in semi-arid soils in a previous study (INGHAM & COLEMAN, 1984), this implies that bacterial populations in the Georgia soils were either less susceptible or more resilient than the semi-arid grassland soil, although the mechanism for this was not investigated. The short-term reduction in bacterial numbers was not enough to result in competitive release of fungal activity in litter or soil, as was observed in the semi-arid prairie study. Flagellate numbers increased in the streptomycin-treated litter without detection of a reduction in bacterial numbers. This suggests that the number of bacteria actually increased in the streptomycin-treated litter, but was rapidly grazed by the flagellate population, stimulating an increase in flagellate numbers.

Addition of streptomycin resulted in an increase in concentrations of both ammonium- and nitrate-N. Streptomycin contains a number of labile amide groups and the increase in inorganic N following streptomycin addition has been observed previously (INGHAM *et al.*,

1986). In the study in semiarid soils, nitrate-N was significantly reduced after streptomycin application, indicating that nitrifying bacteria were especially susceptible to streptomycin. In the Georgia soils, however, nitrate-nitrite-N concentrations were much higher after streptomycin application, suggesting one of the following possible occurrences; the presence of less susceptible populations of nitrifying bacteria, greater protection of nitrifying populations in the higher clay-content soil, or these soils contain fungi capable of nitrifying ammonium-N released from streptomycin.

After streptomycin application to these soils, plants exhibited severe chlorosis, typical of anaerobic conditions in the soil around the root systems. Eventually, all of the plants died, and even a year later, these plots could be easily picked out as a result of poor plant growth. Further investigation of the interaction of bacterial death and the production of conditions in the soil detrimental to plants is needed.

Captan reduced fungal activity in litter throughout the experiment, but reduced fungal activity in soil only on the first two sample dates. As in most soils, total hyphal length did not immediately reflect the reduction in active hyphae. A large quantity of dormant or senescent fungal biomass is often present which masks the more dynamic response of metabolically active hyphae.

Captan indirectly reduced fungal-feeding nematode numbers, evident by the fact first lower active hyphae were observed, followed by the reduction in fungal-feeding nematodes. Thus, a reduction in food resource available for these predators resulted in reduced numbers of fungal-feeding nematodes. If mineralization of N from fungal biomass by fungal-feeding nematodes is an important factor in determining inorganic N levels in soils, lower inorganic N levels should have been observed. However, reduction in fungal biomass, followed by reduction in fungal-feeding nematode numbers had no observable effect on inorganic N pools. Fungi are often capable of net N mineralization (HUNT *et al.*, 1988), such that reduction in fungal activity might reduce mineralization of N as well as reduce immobilization into fungal biomass. In this case, no net effect would be observed with respect to inorganic N levels in soil. Alternatively, plants may have been extremely N-limited such that the turnover in the inorganic-N pool in the soil was extremely rapid, and any mineralized N was rapidly removed. Since a variety of interactions have been observed a number of times in foodweb studies, there is ample reason to investigate N immobilization-mineralization processes with labeled N experiments.

In conclusion, streptomycin was ineffective in reducing its bacterial target group in these soils, and instead resulted in the death of the plants, a non-target group. Captan was again shown to be an effective fungicide with no direct non-target effects.

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Single applications of a bactericide, streptomycin, and a fungicide, captan, were made in a Georgia no-till agroecosystem. Streptomycin treatment of soil resulted in reductions in bacterial populations, but also resulted in the death of plants within a two to four week period, indicating indirect effects on plants mediated by bacteria. Captan was effective in reducing fungal activity, and total fungal biomass, without directly affecting any non-target group. An indirect effect of reducing fungal biomass with captan resulted in a short-term decrease in numbers of fungal-feeding nematodes, as the food resource for this group of predators was reduced.

Keywords: Geogia, no-till agroecosystem, streptomcycin, captan, Bacteria, fungi, activity, biomass, Nematoda, fungivora, microarthropod.

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